

ON EXPERIMENTAL VARIOLA IN THE MONKEY.

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I. INTRODUCTION.

The studies forming the subject of this paper were undertaken in connection with a general investigation into the etiology of variola, at the suggestion of Dr. W. T. Councilman, whose constant guidance and advice in their prosecution we most gratefully acknowledge.

The research was conducted with the following general objects in view: (1) the observation of the course of variola inoculata in the monkey, with especial reference to the constitutional reaction, the specific lesions of the skin, and the disturbances in the leucocyte equilibrium incidental to the disease; (2) the gaining of additional knowledge in regard to the occurrence and the distribution of the etiological factor.

The work was carried on during the past year, for the most part in a small laboratory, temporarily established at the Boston Detention Hospital.

II. HISTORICAL REVIEW.

Experimental inoculation of monkeys has been practised by various investigators engaged in the study of variola, and it is a matter of knowledge that the monkey is an animal susceptible to the disease. Very little attention, however, seems to have been given in the course of such experiments to the histological characteristics of the induced lesions.

Zülzer (1874) variolated a *Cercopithecus* with the blood of a variola patient, producing constitutional symptoms and a general papular eruption. He was also able to produce the disease by exposing the animal to air containing finely divided particles of desiccated crusts wet with pustule contents. His efforts to inoculate through the alimentary tract were unsuccessful.

Buist (1887) found that inoculation of the ape with dry beer yeast produced the same reaction as that yielded by inoculation with vaccine and with variola lymph, and further

that animals so treated displayed a diminished susceptibility to vaccinia and to variola.

Copeman (1894) demonstrated the susceptibility of *Rhœsus* alike to vaccinia and to variola, finding that in this animal the local reaction to both diseases first appears on the third day, that the vesicle formation is complete on the fifth, and that the maximum development of the lesion is attained upon the eighth day. He found the lesion to be less marked in vaccinia than in variola.

De Haan (1896) inoculated *Macacus* with the contents of variola pustules, obtaining on the seventh day local lesions in the form of papules surrounded by an areola. In one of a series of seven cases, he observed a few secondary papules upon the lips and upon the extremities.

Roger and Weil (1902) report a series of experiments upon *Macacus*. Inoculating with variola pus, they obtained pustules which dried up in fifteen days. Inoculations made by them beneath the skin produced no lesions. In a series of inoculations made with the blood of cases of hemorrhagic variola, they obtained pustule formation in an animal inoculated by scarification, and no lesions when inoculation was performed by injection of the blood into the animal. One animal subjected to the latter procedure died on the fifth day from streptococcus septicemia. These investigators also injected two monkeys with blood from a rabbit inoculated four days previously with variola pus. The animals showed typical changes in their leucocytes, but derived no immunity to vaccinia. These writers regard both vaccinia and variola as inoculable upon the monkey, but hold that neither confers perfect immunity.

Park (1902) found Java monkeys susceptible to variola and insusceptible to varicella.

Ewing, in the same year, reports that he produced typical variola lesions in *Rhœsus* and in an African monkey.

III. METHOD.

The animals used in these experiments were small monkeys, belonging to the *Rhœsus* and to the *Macacus* groups.

The experiments are presented in three series, in each of which the virus employed was from a different local epidemic. In the first series particular attention was paid to the constitutional reactions and the conditions of the blood, in the second and the third series attention was directed to the study of the evolution of the lesion.

Series I. — Prior to inoculation, each animal was subjected to a preliminary control observation, consisting in the determination of body temperature, the making of blood counts, and the microscopic examination of the freshly drawn blood. Temperature was taken per rectum. Blood was derived from small incisions made with aseptic precautions, either in the tail or within a shaved area upon the back. Microscopic examination of the fresh blood was conducted upon the warm stage, at body temperature, and by means of a Zeiss apochromatic optical series.

Series II. and III. — The general condition of the animal, the temperature, and the macroscopic appearance of the lesions were noted daily.

The method usually employed for introducing the virus into the animal was that of local inoculation of the skin. In one experiment intravenous injection was practised. The regions selected for inoculation were the upper arm, the abdomen, and the back between the scapulæ. Inoculation was performed by scarifying or by lightly incising the skin, previously shaved and rendered aseptic by washing with alcohol and rubbing in with a flamed knife blade the infective material.

Three strains of virus were used in these several series of experiments. Through the courtesy of the health authorities in Fall River, Mass., and in Philadelphia, Penn., we were able to obtain virus from cases occurring in local epidemics in these cities. The experiments are grouped according to the strain of virus inoculated. The samples of virus from the different individuals are indicated by a letter after the Roman numeral which designates the epidemic strain to which the virus belongs. Thus Virus I. *a* and Virus I. *b* are from the same epidemic, but from different cases in

that epidemic. The strains are numbered as follows: Virus I., Boston epidemic; Virus II., Fall River epidemic; Virus III., Philadelphia epidemic. The virus was obtained by drawing the contents of a vesicle or pustule into a small sterile capillary tube. The material was used within twenty-four hours after collection. In certain experiments other infectious material was employed, such as powdered disks, or a paste made of the same. In many cases the material used for inoculation was tested on the rabbit's cornea.

Such animals as died or as were killed were promptly subjected to post-mortem examination, and their tissues preserved in appropriate fixatives. In certain cases corneal inoculations were practised with scrapings from the specific lesions or with material from certain organs of the animal in order to demonstrate the presence of the contagium. The tissues derived from autopsy were fixed in Zenker's fluid, embedded and cut in paraffine, and stained by a variety of methods. The most satisfactory results were obtained with the eosin-methylene blue stain. Bacteriological examinations at autopsy were made in appropriate cases.

SERIES I. — EXPERIMENT NO. I.

Animal a young male *Macacus*. — Preliminary injection with 0.002 gram Koch's tuberculin, followed by rise in temperature of about one degree during the subsequent forty-eight hours. On the second day following the tuberculin test, inoculation was made by rubbing into scarifications upon the shaved skin of the upper arm freshly-ground disk from a case of variola vera.

Temperature, already elevated about one degree above the normal for the animal, at 37° C., rose to 38°, remained at that point for one day, then fell to normal, rising again to 37° on the fifth day, and subsequently falling to an ante-mortem subnormal point of 34°.

The site of inoculation showed no inflammatory reaction, and the scarifications gradually healed, with no evidence of "take."

Constitutional symptoms of a specific character were wanting; the animal developed a dyspnea, and grew progressively weaker, dying upon the eleventh day after the inoculation.

Autopsy showed tuberculosis of the bronchial lymph nodes; these were greatly enlarged, and impinged upon and constricted the primary bronchi.

The red blood corpuscle count varied from about six and one-half millions

per cubic millimeter at the beginning of the experiment to rather less than five millions at the death of the animal.

The leucocytes showed an increase to 23,000 per cubic millimeter on the second day after inoculation, followed by a decrease to about 10,000 during the last week of life.

Histological examination of the skin at the site of inoculation showed slight evidence of repair, but no specific lesion.

Experiment negative.

SERIES I. — EXPERIMENT NO. 2.

Animal a young male *Macacus*. — Inoculated with vesicle contents from a case of variola vera, obtained in a capillary tube, and rubbed into a scarified area upon the arm. Under observation nineteen days (*Virus I. a.*).

Temperature rose a degree during the first twenty-four hours after inoculation, returning to normal upon the fourth day. On the fifth day the area of scarification showed well-marked induration and a faint, peripheral flush; under a hand lens distinct, transparent, nodular elevations were apparent. The temperature at this time showed a rise of two degrees to 38° C., and the animal displayed some restlessness and anorexia. During the next three or four days the local lesion gradually developed, showing on the ninth day well-marked vesicles, strongly elevated, confluent, and partially desiccated. At this time the temperature rose to 39°, three degrees above the normal. Following this was a gradual subsidence of the lesions, with desquamation of one of the crusts on the eleventh day. The temperature slowly returned to normal. On the sixteenth day the site of inoculation showed two dry, scaly crusts.

The red blood corpuscle count showed fluctuations between three and six millions per cubic millimeter from the fifth to the ninth days.

The white corpuscle count showed a leucocytosis of 26,000 per cubic millimeter on the third day after inoculation, and another of similar degree upon the tenth day persisting for three days. This was mainly a mononucleosis.

Nine days after the healing of the local lesion re-inoculation was attempted; material derived from scraping the base of a protopustule¹ of the animal, the subject of Exp. No. 3, was rubbed into scarifications upon the front of the chest and upon the back. Upon the third and the fourth days following this procedure the animal showed a slight rise of temperature, accompanied by a moderate polynucleosis; the site of inoculation showed a little induration, which soon subsided, and upon the sixth day was healed. Upon the twelfth day after the attempted re-inoculation the animal was vaccinated upon the back with points giving a positive reaction upon the rabbit's cornea. This vaccination failed to take. Animal allowed to survive.

¹ The term "protopustule" (Pfeiffer) is used to designate the lesion developed at the site of inoculation.

Result of the experiment.—Production of variola inoculata (Virus I. *a*), with local lesion, the latter apparent on the fifth day, becoming fully developed on the ninth day, and gradually subsiding with final desquamation by crusts on the sixteenth day; fever from the fifth to the fourteenth day; leucocytosis on the third, and from the tenth to the nineteenth days. Immunity to re-inoculation (Virus I. *b*) and to vaccination.

SERIES I. — EXPERIMENT NO. 3.

Animal a young male *Macacus*. — Inoculated upon the belly with vesicle contents from a mild case of variola vera (Virus I. *b*). Temperature rose on the third day to 39.3° C. On the fourth day the site of inoculation showed evidence of reaction. At this time a second inoculation was performed with the same material as that employed in the first (Virus I. *b*). Temperature slowly fell during the third, fourth, and fifth days, rising again to 39.5° C. on the sixth day, forty-eight hours after the second inoculation, gradually returning to normal on the eleventh day. On the fourth day the animal showed marked constitutional reaction, with anorexia. This was again apparent upon the eighth day, the animal seeming weak and depressed. At this time the site of the first inoculation presented two areas one centimeter across and two to three millimeters in elevation, indurated, red, the center depressed and bearing a small, irregular crust; within one of these areas was a shotty papule. The second inoculation showed elevation and redness, with a few minute vesicles.

No observations upon the erythrocyte content of the blood were made during this experiment.

The white blood corpuscle count showed at the end of forty-eight hours after each inoculation a leucocytosis of about 30,000; differential count proved this to be essentially a polynucleosis, although throughout the experiment there was a gradual increase in the mononuclear elements of the blood.

Upon the eleventh day the animal was chloroformed. Autopsy showed nothing abnormal in the internal organs. Histological examination showed some cloudy swelling and fatty metamorphosis of the kidneys, proliferation of phagocytic cells in the axillary lymph nodes. The skin at the site of the first inoculation (protopustule eleven days after inoculation, seven days' development) showed crust formation, with superficial necrosis and degeneration of the epithelium beneath its middle portion; the epithelium contiguous to the edges of the crust showed some proliferation; no parasites were demonstrable in the newly-formed cells (Plate XVII., Fig. 2).

Result of the experiment. — Production of variola inoculata, two infections four days apart (Virus I. *b*); constitutional reaction; leucocytosis. No immunity from the first inoculation on the fourth day.

SERIES I. — EXPERIMENT NO. 4.

Animal a young female *Macacus*. — Inoculation upon two scarified areas on the abdomen with vesicle contents from a case of variola vera (Virus

I. *c*), subsequently proven to be of the abortive type. A second inoculation was made at the same time by linear incision upon the back, between the shoulders. The temperature rose in the following forty-eight hours two degrees to 39.5° C. On the fourth day the abdominal inoculation showed slight induration, peripheral flush, and a small central crust. On the fifth day both sites of inoculation showed well-marked reaction. The temperature was continuously elevated at 39° and 39.5°. Animal presented no constitutional symptoms. On the seventh day, numerous red, shotty papules appeared upon a shaved area of the back used for obtaining blood. Multiple lesions of the same general character were scattered over the trunk and the head. At this time curettings from one of the abdominal pocks were inoculated upon the rabbit's cornea with positive results.

White corpuscle count showed a very slight rise on the fourth day, followed by a somewhat more marked leucocytosis upon the seventh and the eighth days; differential count showed this increase to be shared in about equally by poly and by mononuclear elements.

Upon the eighth day after inoculation, and the fifth after the appearance of a local lesion, the animal was chloroformed.

Autopsy showed, in addition to the protopustules, about forty skin lesions in the form of nodular elevations, one to three millimeters in diameter, and distributed widely over the head, trunk, and extremities; all of these lesions were firm and shotty, the larger somewhat yellowish. The internal organs were normal.

Histological examination of the protopustule showed an extensive superficial crust, the tissue beneath which was infiltrated with leucocytes, the cells of the hair follicles at the edge of the lesion and those of the adjacent deep layers of the epidermis containing the variola parasite. The lesions of the general exanthem showed histologically a collection of leucocytes in and beneath the epidermis, together with necrosis, the cells of the included and contiguous hair follicles containing the parasite (Plate XXVII., Fig. 4).

Result of the experiment. — Production of variola inoculata, with protopustule and general exanthem (Virus I. *c*). Specificity proven by presence of organism.

SERIES I. — EXPERIMENT NO. 5.

Animal a full-grown female Rhæsus. — Inoculation by injection into the anterior jugular vein of a suspension in normal saline solution of about one cubic centimeter of the contents of a pustule from a case of variola vera (Virus I. *d*). Contents of the same pustule inoculated upon the rabbit's cornea gave a specific reaction. Upon the following day a large area of the back was closely shaved. Temperature at this time was subnormal; the animal seemed sick and refused to eat. Leucocytes 21,000 per cubic millimeter.

Upon the second day after inoculation the animal died. Autopsy showed between the muscles at the site of the incision in the neck thin, sanguineous fluid; smear from this exhibited many polynuclear leucocytes

but no bacteria. The skin presented no eruption of any sort. Of the internal organs, the left lung showed a small patch of broncho-pneumonia; the liver, extensive central necrosis with hemorrhage; the kidneys, marked cloudy swelling and fatty metamorphosis. Bacteriological examination showed streptococcus in the heart's blood in pure culture, and in the wound of the neck a variety of other organisms. Histologically, the lung contained within the pneumonic area short chains of streptococci. No changes were present in the skin.

Result of the experiment. — *Streptococcus septicemia*; death in forty-five hours.

SERIES I. — EXPERIMENT NO. 6.

Animal a full-grown male *Rhæsus*. — Inoculation upon a scarified area of the back with contents of a pustule from a case of *variola vera* which gave a positive reaction upon the cornea of the rabbit (*Virus I. d*). Temperature rose upon the following day one degree to 39° C., remaining at this point until the fifth day, when it increased to 40.3°. Upon the fourth day after inoculation the site of inoculation showed multiple areas somewhat elevated and indurated. No marked constitutional symptoms, the animal natural and eating well. Upon the sixth day small, secondary papules appeared in the immediate vicinity of the primary local lesions. Upon this day the animal was chloroformed.

White count showed a leucocytosis of 30,000 present before the beginning of the experiment, differential count showing this to be a polynucleosis; a fall in the leucocyte content occurred from the second to the fourth days, followed by a slight rise to about 15,000 on the fifth and sixth days, the rise mainly polynuclear.

Autopsy showed a normal condition of the internal organs. Cultures from the heart, liver, spleen, and kidney showed no growth.

Histological examination of the protopustule of the skin, sixth day after inoculation, the second of its development, showed a superficial crust, bordered on either hand by proliferated epithelium, and underlaid by an area of corium in which were hair follicles and glands in various stages of degeneration and diffuse infiltration with various migratory cells. The epithelial cells of the follicles under the center of the lesion, and those more towards the border of the lesion and in the adjacent epidermis, showed the *variola* organism.

Result of the experiment. — Production of *variola* inoculata with fever, but no apparent constitutional reaction (*Virus I. d*); specificity of inoculation proven by the presence of the *variola* organism in the protopustule.

SERIES II. — EXPERIMENT NO. I.

Animal a young male *Rhæsus*. — Multiple, light incisions on the skin of the abdomen were inoculated with a paste made by grinding up with sterile salt solution a number of eight months old disks from a case of confluent *variola vera*. At the same time a rabbit was inoculated on both corneas with the material, which produced a typical variolous keratitis. The monkey showed a slight transient rise of temperature on the

second day after the inoculation. No constitutional symptoms were noted. The inoculation sites showed no reaction save those incident to repair of the slight incisions. On the fifth day after this unsuccessful inoculation with disk paste a fresh area on the skin of the abdomen was inoculated with virus from a mild case of variola vera (Virus II. *a*). During the succeeding seven days the temperature ranged between 37.8° and 39° C. The inoculation sites showed, on the second day, slight elevation without redness. On the fourth day the lesion exhibited, in addition to the above, a peripheral flush. On the seventh day there was a well-developed central crust bordered by a pale elevation which merged peripherally into a narrow zone of redness. The axillary lymph nodes were distinctly enlarged during the active evolution of the lesion, and that on the right side was tender. No constitutional symptoms were observed. The lesions in this animal did not attain the size of those in the animals of the preceding experiments where Virus I. was used. On the seventh day after inoculation the animal was chloroformed and an autopsy done at once.

Autopsy. — Skin lesions as above. Considerable edema of the areola tissue beneath the inoculations. Axillary lymph nodes enlarged and red. On section the cut surface yields turbid blood-stained fluid. All internal organs appear normal.

Histological examination. — Inoculation sites. — The epidermis presents a lenticular cavity divided into smaller, irregular spaces by strands of compressed and degenerated epithelial cells. The vesicle is bounded above by cornified epithelium and inspissated exudate, below by the more or less degenerated cells of the rete, and at the sides by proliferated epithelial cells. Polynuclear leucocytes are present in large numbers in the vesicle, in the surrounding epithelium, and in the underlying tissue. The variola organism is present in the cells bordering on the vesicle.

Axillary lymph nodes. — The sinuses contain many lymphoid cells and phagocytes. The latter frequently include red corpuscles in their protoplasm. Inguinal and mesenteric lymph nodes normal. Lungs, liver, spleen, kidney, testicle, and bone marrow examined and found normal.

Result of the experiment. — Failure to produce a lesion by inoculation with "disk paste" which did produce a variolous keratitis in the rabbit. Subsequent production of a mild protopustule by inoculation with Virus II. *a*. Specificity of lesion proven by finding organism in the epithelial cells of the lesion.

SERIES II. — EXPERIMENT NO. 2.

Animal a young male Rhæsus. — Inoculated in six places on the skin of the abdomen with virus from a case of variola vera (Virus II. *b*). Lesions developed which were identical with those of the previous experiment. The animal was allowed to survive. Nine days after the first inoculation the procedure was repeated, this time with a new strain of virus (Virus III. *a*). This virus was clear vesicle contents from a case of mild variola vera. No reaction followed the second inoculation. The

potency of the virus was shown by the results of Experiments III. 1 and III. 2, in which the same was used.

SERIES II. — EXPERIMENT NO. 3.

Animal a young male Rhesus. — Inoculated at the same times and with the same materials as the previous animal (Experiment 2).

Result of the experiments. — Successful inoculation of both animals with Virus II. *b* with the production of a mild protopustule. Development on the ninth day after inoculation of an immunity to a virus of proved virulence. Specificity of lesion proven by immunity.

SERIES III. — EXPERIMENT NO. 1.

Animal a young male Rhesus. — Inoculated on the abdomen with virus from a case of mild variola vera (Virus III. *a*). The temperature rose to 39° C. on the third day after inoculation, reaching 40° C. on the fourth and fifth days. On the second day the sites of the inoculations were elevated and indurated, but showed no change of color. The next day a pink flush was seen about the lesions, and the axillary lymph nodes were enlarged. On the two succeeding days the lesions became larger, the center exhibited a crust bordered by elevated epithelium, surrounded in turn by a red areola. On the fifth day the individual lesions measured eight millimeters across, and the axillary lymph nodes were enlarged and tender. The animal was chloroformed and an autopsy made at once.

Autopsy. — Skin lesions as above. The areolar tissue beneath the lesions edematous. Axillary lymph nodes enlarged and red. On section a turbid, blood-stained fluid flows readily from the cut surface. Inguinal lymph nodes normal. All internal organs appear normal.

Histological examination. — Skin lesions. — There is a narrow crust beneath which is a cavity containing finely granular material, in which are a moderate number of polymorphonuclear leucocytes and of degenerated epithelial cells, together with a loose network of fibrin. The vesicle is bounded laterally by proliferated epithelium, and below by the more or less degenerated cells of the rete. There is a moderate infiltration of the corium and of the epithelium adjacent to the vesicle, with polymorphonuclear leucocytes. Many stages of the variola organism are present, both protoplasmic and nuclear forms being represented (Plate XXVII., Fig. 1).

Lungs, spleen, liver, kidney, testicle, and bone marrow show no lesions. The sinuses of the axillary lymph nodes contain many endothelial cells which are frequently phagocytic. The inclusions are, in the main, red blood corpuscles.

Result of the experiment. — Production of protopustule by inoculation with Virus III. *a*. Specificity of the lesion proven by the finding of the organism.

SERIES III. — EXPERIMENT NO. 2.

Animal a young female Rhæsus. — Inoculated on the skin of the abdomen with vesicle contents from a mild case of variola vera (Virus III. *a*). On the second day after the inoculation the temperature rose to 39° C., and ranged between that and 40.6° C., until the end of the experiment. On the second day after the inoculation the incisions showed a slight elevation with a faint, pink flush. On the succeeding days the lesions enlarged, showing a depressed crust on the fifth day, at which time the elevated margin and the red areola were most marked. After the eighth day the lesions did not increase in size. From the fourth day onward the axillary lymph nodes were enlarged and tender. On the eighth day two shotty nodules were noted, close together, and one centimeter from the primary lesion, in the direction of the left axilla. Two small vesicles were present on the anterior aspect of the pubes, one on the back, in the lumbar region, one on the inner aspect of the right arm, one on the inner aspect of the left fore-arm, and two on the lower jaw. In all, nine papules or vesicles were found. The animal was chloroformed the next day, the ninth after inoculation, and an autopsy made at once. There had been no further development of the exanthem, and the primary lesions had not enlarged.

Autopsy. — Skin lesions as above. Rabbit's cornea, inoculated from the primary lesions, developed a typical variolous keratitis. Axillary lymph nodes enlarged and red. On section, the surface yields turbid, blood-stained fluid. Rabbits' corneas inoculated from scrapings of the node showed no reaction. Inguinal lymph nodes normal. Lungs show advanced tuberculosis with cavity formation. Bronchial lymph nodes, spleen, and kidneys also the seat of tuberculosis. Rabbit's cornea inoculated with scrapings from the spleen pulp showed no reaction. Other organs appear normal.

Histological examination. — Primary lesion presents a thick crust lying upon the corium. On either side the epidermis is thickened, and in the cells are various stages of the variola parasite. The tissue of the corium is infiltrated with leucocytes, and the epithelial cells of the sheaths of the included hair follicles are more or less degenerated, and also contain the variola parasite.

Exanthem presents a small crust bordered by proliferated epithelium. Beneath the crust is a small mass of polymorphonuclear leucocytes, which cells are also infiltrating the adjacent corium. The variola parasite is present in the epithelial cells at the edge of the lesion.

Axillary lymph nodes. — Sinuses contain many phagocytic cells, which include both polymorphonuclear leucocytes and red blood corpuscles. Inguinal and mesenteric lymph nodes normal. Lungs, spleen, and kidneys show tuberculosis. Liver presents fatty infiltration at the periphery of the lobules. Bone marrow and testicle normal.

Result of the experiment. — Production of protopustule followed by a general eruption, as the result of inoculation with Virus III. *a*. Specificity

of lesions shown by the presence of the variola parasite and by corneal inoculations. Failure to demonstrate the contagium in the axillary lymph nodes and in the spleen pulp.

SERIES III. — EXPERIMENT NO. 3.

In this experiment a virus was used having the following history: A rabbit's cornea was inoculated with scrapings from the primary lesion of the animal in Experiment 2 of this series. The inoculation was made on the ninth day of the evolution of the lesion. A typical variolous keratitis developed. After forty-eight hours part of the lesion was scraped from the cornea and divided into two portions. One portion was inoculated on the skin of a calf, but produced no lesion. The second portion was inoculated on the cornea of a second rabbit producing a typical variolous keratitis. After seventy-two hours a part of the lesion of this second rabbit was scraped off and transferred to the cornea of a third rabbit, producing there a typical variolous keratitis. After seventy-two hours this lesion on the cornea of the third rabbit was transferred to the cornea of a fourth rabbit, producing a typical variolous keratitis. After seventy-two hours the lesion on the cornea of this fourth rabbit was scraped off and divided into two portions. One portion was transferred to the cornea of a fifth rabbit, producing a typical variolous keratitis. The second portion was used to inoculate two small scarifications on the skin of the abdomen of a young male "hooded" monkey (Calcutta). Following this inoculation no rise of temperature was noted. The animal took nourishment well until the fifth day, when a slight anorexia was manifest. Coincident with this a profuse diarrhea developed. On the third day after inoculation the scarified areas were pink and slightly elevated. At this time the axillary lymph nodes were palpable. On the succeeding days the lesions enlarged, attaining on the seventh day a diameter of one centimeter, and presenting a depressed central crust bordered by an elevated margin which in turn merged into a red areola. With the development of the lesions, the axillary lymph nodes were notably enlarged and tender. The inguinal lymph nodes remained normal. On the seventh day after inoculation an eruption was observed consisting of five papules and vesicles. These lesions were situated as follows: one on anterior aspect of right thigh, one on right shoulder, one on right cheek, two on lower lip. Besides these cutaneous lesions the upper surface of the tongue presented three circular losses of substance about two millimeters in diameter. The animal was chloroformed and an autopsy done at once.

Autopsy. — Skin lesions as above. Material from primary lesion and from vesicle on the lip inoculated on rabbits' corneas. (In both animals a typical variolous keratitis developed.) On section the areola tissue beneath the primary inoculation edematous. Axillary lymph nodes enlarged and red; on section the surface yields turbid, blood-stained fluid. Scrapings from the surface of section of the node inoculated on a rabbit's cornea. A typical variolous keratitis developed. Inguinal lymph nodes normal. All internal organs appear normal.

Histological examination.—Primary lesion presents a crust bordered by thickened epithellium and underlaid by corium. Many polymorphonuclear leucocytes are invading the tissues about the lesion. The variola parasite is present in the epithelial cells at the edge of the crust. **Exanthem.**—There is a small cavity in the epidermis bounded above by the cornified epithelial cells, below and at the sides by the proliferated epithellium. The vesicle contains many polymorphonuclear leucocytes and much granular precipitate. The variola parasite is present in the epithelial cells about the lesion (Plate XXVII., Fig. 3). **Tongue.**—The cells of a small segment of the epithellium are more or less degenerated, and the tissue there and beneath the lesion is infiltrated with polymorphonuclear leucocytes. Cytoplasmic stages of the organism are present in the epithelial cells. **Axillary lymph nodes.**—The sinuses contain a number of polymorphonuclear leucocytes and of red blood corpuscles. These cells are frequently included in the protoplasm of phagocytic endothelial cells which are present in large numbers. Lungs, liver, and testicle normal.

Result of the experiment.—Production of a protopustule, followed by a general eruption on the seventh day, by inoculation with Virus III. *a* which had been transferred through one monkey and four rabbits. Specificity of lesion proven by corneal inoculation and by the presence of the variola parasite.

IV. DISCUSSION OF RESULTS.

From a review of these experiments it is apparent that positive results were obtained in ten out of twelve. The findings in these ten cases of successful inoculation may be grouped for discussion under the head of: (1) the course of the disease; and (2) the histology of the skin lesions.

1. The course of the disease offers for study the occurrence, the nature, and the degree of constitutional reaction; the character, the evolution, and the specificity of the skin lesions; and the disturbances of the leucocyte equilibrium.

Constitutional reaction was evident mainly by fever; some anorexia and restlessness were apparent in three of the experiments.

Fever began in one case on the first day, in one on the fifth day, and in three cases on the second day after inoculation; reached its maximum from the fourth to the eighth day; and, in those experiments sufficiently prolonged to permit of observation, declined by lysis, temperature becoming normal by the end of the second week. The extent of

the temperature elevation averaged 2.3° C. The period of most marked fever coincided with that of the full development of the local lesion. Anorexia when present was most marked at about the fourth or fifth day.

The specific skin lesions consisted in a primary pock (protopustule), developed at the site of inoculation, and present in all ten of the cases; in secondary lesions, developed in the vicinity of the primary, present in two cases; and in a general papular eruption, in addition to the primary lesion, present in three cases.

The primary lesion was first evident upon the third day. In some animals no reaction was observed till the fifth day. Its full evolution was observed in four experiments. It is first evidenced by induration of the site of inoculation, the edges of the indurated region showing a faint pink color. The lesion gradually increases in extent and in elevation, reaching maximum development by about the eighth day, when it presents as a hemispherical, elevated, indurated area, from one to three centimeters in diameter, the center bearing the crust is surrounded by a pink areola. At a somewhat earlier stage, small vesicles containing clear fluid may be detected at the center of the lesion. The swelling gradually subsides, the vesicles dry up, and by the end of the second week desquamation of the primary crusts takes place.

The specificity of the skin lesions may be asserted upon the basis of a variety of data. Thus: Immunity to variola, to vaccinia, or to both conferred by inoculation; positive reactions when curettings from the lesions were inoculated on the rabbit's cornea; the presence of the variola organism on histological examination.

Disturbance in leucocyte equilibrium is manifest in the course of the disease. It seems probable that a transitory leucocytosis upon the second or third day after the inoculation, followed by a second leucocytosis at about the height of the development of the local lesion, is characteristic. These conditions prevailed in two experiments. There are good reasons for believing that in the second leucocytosis

mononuclear elements play an important part, a condition, which, if true, may, perhaps, be interpreted as indicative of disturbance in cell differentiation in the bone marrow. We regard our data upon this point as inadequate for generalization.

2. The histological study of the specific lesions of the skin brought out points of difference, structurally, between the lesions of the exanthem in man and the analogous lesions in the monkey. The lesions of the monkey differ from those in man principally in that more polymorphonuclear leucocytes are present. The structure of the vesicle and of the pustule is developed at the site of inoculation practically the same as that of the lesions of the exanthem in man in variola vera. The lesions of the exanthem in the monkey resemble most closely the skin lesions in man seen in abortive variola. The process of healing differed in no wise from that observed in man. The minute study of the lesion, with respect to the presence and distribution of the etiological factor, forms the subject of another paper.

One animal was shown to be immune to both variola and vaccinia after successful inoculation with Virus I. Two animals were shown to be immune to variola (Virus III.) after inoculation with Virus II. These results are in keeping with those of other investigators.

The comparison of the lesions produced by inoculation with the different strains of virus from different epidemics is of especial interest, and seems to us to be suggestive. The mild lesions produced by inoculation with Virus II. contrasted sharply with the severe ones which followed inoculation with Virus I. or Virus III. The epidemic from which Virus II. was obtained was of a clinically mild type of the disease, while the epidemics from which Virus I. and Virus III. were obtained were severe.

A generalized eruption was observed three times in the eight animals who were successfully inoculated, and who were allowed to survive long enough to show the eruption. Of these eight animals three were inoculated with Virus II., and none showed a general eruption, while of the remaining

five monkeys inoculated with Virus I. or Virus III., three developed the exanthem.

In two instances the monkeys failed to react to inoculation with the variola disk. In one of these cases the animal was subsequently shown to be susceptible to variola. The disk used was active when inoculated on the rabbit's cornea. We are unable to explain these results, and propose to make this the subject of further investigation.

The results obtained in the last experiment (Series III., No. 3), where a monkey was successfully inoculated with a virus that had been transferred from one rabbit's cornea to another for four generations, indicates a line of work that promises much. If the development of an exanthem in the monkey postulates variola rather than vaccinia, we have succeeded in passing variola through the tissues of the rabbit, a herbivorous animal, without its becoming vaccinia.

In one experiment, by corneal inoculation on the rabbit, the presence of the contagium was demonstrated in the axillary lymph node.

V. CONCLUSIONS.

1. *Macacus* and *Rhesus* monkeys are susceptible to variola inoculata.

2. The disease in these animals pursues a typical course, with more or less constitutional reaction and with fever, and presents lesions of the skin consisting ordinarily of a primary pock at the site of inoculation, sometimes with local secondary lesions, and less commonly with an associated general exanthem.

3. The disease is accompanied by a disturbance in leucocyte equilibrium characterized by a polymorphonuclear, followed by a mononuclear leucocytosis, the full significance of which cannot be interpreted from the amount of data at our disposal.

4. The disease produced in the monkey by inoculation with variola virus is not identical with variola vera in man; it agrees with variola inoculata in man.

5. The lesions contain, within the epithelial cells of the epidermis and of the hair follicles, *Cytoryctes variolæ*.

6. Successful inoculation with variola confers immunity to subsequent inoculation with variola or with vaccinia.

7. Different strains of virus from different epidemics exhibit different degrees of virulence for the monkey. Virus from epidemics of clinically severe variola produces more severe lesions and is more likely to be followed by a general eruption. No differences were noted in the virulence of different strains of virus from cases of varying severity in the same epidemic.

8. Variola virus can be transferred from man to a monkey, from the monkey to the rabbit's cornea through four generations, and when then inoculated on the monkey can produce a protopustule followed by a general exanthem.

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DESCRIPTION OF PLATE.

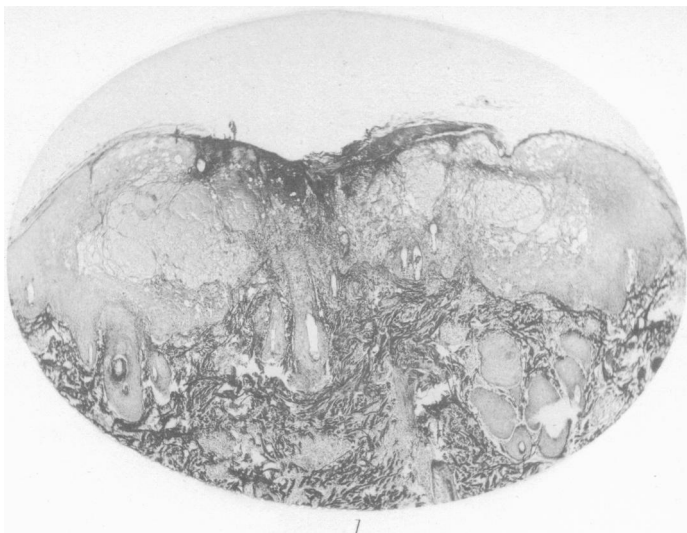
EXPLANATION OF PLATE XXVII.

FIG. 1. Site of inoculation, fifth day of disease. Experiment No. 1, Series III.

FIG. 2. Site of inoculation, eleventh day of disease. The epidermis has grown under the crust. Experiment No. 3, Series I.

FIG. 3. Exanthem, first day of its development, seventh day of disease. Experiment No. 3, Series III.

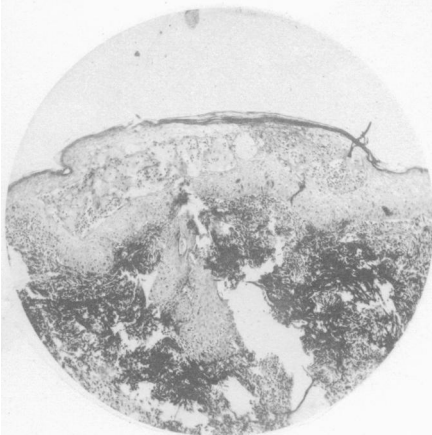
FIG. 4. Exanthem, second day of its development, eighth day of the disease. Experiment No. 2, Series III.



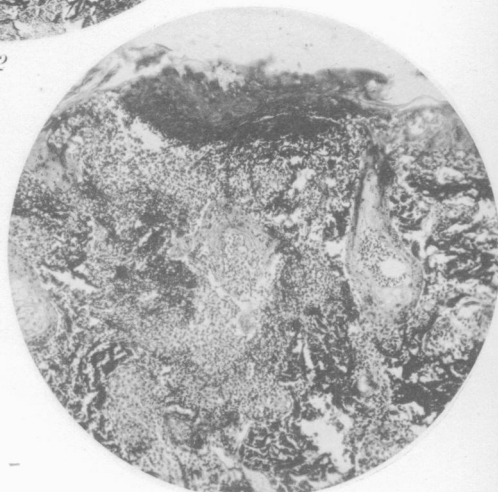
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4

Magrath and Brinckerhoff

Small-pox